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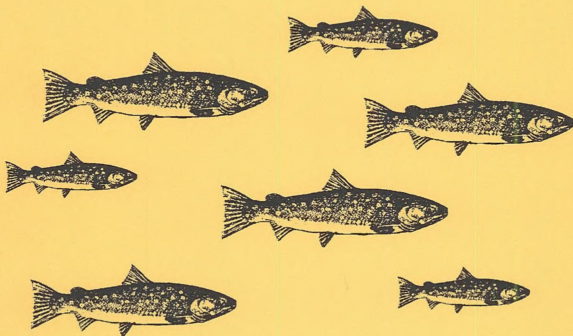

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by

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Mitochondrial DNA Diversity in Bull Trout from the Columbia River Basin

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Running Title: MTDNA VARIATION AMONG BULL TROUT

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Abstract

Bull trout, *Salvelinus confluentus*, a recently recognized species with a broad distribution throughout interior western North America, have undergone dramatic reductions in distributions and population sizes as a result of habitat degradation, introduction of non-native salmonids, and past fisheries management practices. Presently, bull trout are a species of special concern throughout most of their remaining distribution, and conservation of bull trout is now an objective of many state, provincial, and federal agencies.

We used restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) to determine the genetic structure and identify evolutionarily significant units of bull trout in the Columbia River drainage. We amplified approximately 48% of the bull trout mitochondrial genome (the ND-1, ND-2, ND-5/6, and Cyt B regions) using the polymerase chain reaction. Restriction digests of amplified regions revealed variation that allowed separation of bull trout into three distinct clades: Klamath River, lower Columbia River, and upper Columbia River. Clades differed from one another by approximately 1.0 - 1.5% sequence divergence. Bull trout in the upper Columbia River exhibited two very different patterns of mtDNA diversity. Most populations exhibited low mtDNA diversity and shared a common mtDNA haplotype, whereas, two populations from the Lake Pend Oreille system and one population from the St. Joe River exhibited high within-population mtDNA diversity and a low frequency of the common upper Columbia River mtDNA haplotype. The unexpected high levels of mtDNA diversity observed in the Lake Pend Oreille populations suggest that the upper Columbia River Basin and/or large lake systems in that region may contain bull trout populations that incorporate a substantial portion of the remaining natural genetic diversity in the bull trout species.

Bull trout (*Salvelinus confluentus*), a recently described species (Cavender 1978; Haas and McPhail 1991), are primarily an inland char distributed throughout the Intermountain West from northern California, where they are thought to be extirpated (Williams et al. 1989; Moyle and Williams 1990), to the upper Yukon and MacKensie drainages in Canada. Bull trout are largely peripatric and occasionally sympatric with the coastally distributed dolly varden (*S. malma*). Bull trout numbers have declined dramatically throughout much of its range due to habitat degradation, blockage of spawning migrations by construction of dams, misguided fisheries management practices including eradication efforts (Boag 1987), and the introduction of non-native salmonids. Brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) are thought to have replaced bull trout populations through competitive displacement; whereas, brook trout (*Salvelinus fontinalis*) can adversely affect bull trout populations through competitive displacement as well as extensive hybridization (Leary et al. 1983; 1985a; 1985b; Ferguson 1990; Markle 1992).

Bull trout are a species of special concern throughout most of the Intermountain West and Pacific Northwest (Williams et al. 1989). Their status was recently reviewed by the U. S. Fish and Wildlife Service (USFWS) under a petition for listing under the United States Endangered Species Act. The USFWS took the interesting stance of declaring the petition as warranted, but precluded for listing. Thus, presently bull trout are not listed, however, further declines could lead to a second status review and possible listing.

Little is known about the ecology and life history of bull trout; consequently, management agencies are interested not only in preserving remaining bull trout populations, but also in elucidating basic life history information, and determining the extent and structure of residual genetic variation. Such information can lead to

rational management plans that preserve existing ecological and genetic diversity (Allendorf and Leary 1988; Meffe and Vrijenhoek 1988; Quattro and Vrijenhoek 1989; Wayne et al. 1992). In this paper, we examine genetic and geographic aspects of mitochondrial DNA variation among bull trout in the Columbia and Klamath River drainages and relate this to historical biogeography and life history patterns.

Methods and Materials

Bull trout were collected from 15 locations throughout the Columbia River Basin and two locations from the Klamath River Basin (Table 1; Figure 1). Specimens from populations 1-12 (Table 1) were previously examined by Leary et al. (1993) for allozyme variation at 51 putative protein loci. All specimens came from wild populations, except for those from the Clark Fork River and South Gold Creek, which were hatchery produced progeny of wild fish captured at each location. Progeny from the Clark Fork River sample were derived from three females and two males, while progeny from the South Gold Creek sample came from eight females and an unknown number of males (Joe Chapman, Idaho Department of Fish and Game, personal communication).

We isolated mitochondrial DNA from frozen liver or muscle by high speed centrifugation, followed by phenol and phenol/ chloroform/ isoamyl alcohol (25:24:1; vol/vol/vol) extractions. DNA was precipitated by two volumes of 100% ethanol, air dried, and resuspended in 65 µl of sterile distilled water.

Polymerase chain reaction (PCR) was used to amplify four regions of the mitochondrial genome from each individual. These regions were: NADH-1 (hereafter ND1), ND2, ND5/6, and CytB. Primers were provided by LGL Ecological Genetics, Inc. (Bryan Texas). Sequences for the ND1 and ND5/6 primers are described by

Cronin et al (1993). The ND1 primers, 381 and 563B, amplify a mtDNA fragment of 2.0 kb; the ND2 primers, 452 and 562, generate a 2.2 kb fragment; the ND5/6 primers, 763 and 764, generate a 2.4 kb fragment; and the CytB primers, 765 and 766, amplify a 1.3 kb fragment. Amplification was performed in a 40 µl reaction containing: 100 ng of DNA, 8 pmol of each primer, 4 µl of a 10 mM dNTP mix, 4 µl of 10X reaction buffer (Perkin-Elmer), and 0.1 µl of Taq polymerase (Perkin-Elmer). Each PCR cycle (Ericomp or Perkin-Elmer thermal cycler) consisted of denaturation for 50 seconds at 94°C, annealing for 55 seconds at 55°C, and elongation for 2.5 minutes at 72°C. This cycle was repeated 38 times.

Aliquots of each amplified fragment were digested separately with each of ten restriction endonucleases: *Alu* I, *Cfo* I, *Dde* I, *Hae* III, *Hinf* I, *Hpa* II, *Mbo* I, *Msp* I, *Rsa* I, and *Sau* 3AI. *Dde* I and *Hinf* I are pentanucleotide sequence endonucleases while the remainder are tetranucleotide sequence endonucleases. *Mbo* I and *Msp* I are methylation sensitive isoschizomers of *Sau*3A I and *Hpa* II, respectively. Restriction digests were performed in 10 µl reaction volumes according to protocols from the supplier (Promega). Restriction fragments were electrophoretically separated in 3% agarose gels containing ethidium bromide in 40 mM TAE buffer (pH 8). The DNA fragments were visualized by UV transillumination and photographed.

Alphabetic designations were assigned to each unique band pattern generated by restriction endonuclease digests of each amplified mtDNA region. Unique restriction fragment length polymorphisms (RFLP's) among the bull trout populations and a brook trout outgroup were scored with the brook trout pattern arbitrarily designated A, and all other divergent patterns given a different alphabetic designation (Table 2). Fragment patterns (Appendix 1) were converted to restriction site data (presence or absence of site) after log transformation of digitized

measurements of fragment bands and construction of restriction site maps (Appendix 2). REAP (Restriction Enzyme Analysis Package; McElroy et al. 1991) was used to create a binary data matrix (presence or absence of specific restriction sites) for all restriction enzymes and PCR amplified mitochondrial regions. This data matrix was used in PAUP (Phylogenetic Analysis Using Parsimony; Swofford 1993) to generate a set of the most parsimonious relationships among bull trout mitochondrial DNA haplotypes. REAP was also used to produce a diagonal matrix of Nei's (Nei 1987) genetic distances, which in turn was input into PHYLIP (Phylogenetic Inference Package; Felsenstein 1991) where distance methods (KITSCH) were used to estimate relationships among bull trout mtDNA haplotypes.

Results

The amplified fragments of the ND1, ND2, ND5/6, and CytB regions of mtDNA from Columbia and Klamath River bull trout included approximately 48% of the entire mtDNA genome. Restriction sites (N = 310) represent an assessment of 5% of the mtDNA nucleotide sequence. Among bull trout populations and the outgroup brook trout sample, sites were conserved in the ND1 region for the endonucleases *Hae* III, *Hinf* I, and *Msp* I, and in the ND2 region for *Cfo* I (Table 2). At another twenty of the forty enzyme/mtDNA region combinations (10 restriction endonucleases for each of four amplified regions), bull trout populations showed no variation, but differed from brook trout. Thus, bull trout populations exhibited no variation at 60% (24 of 40) of the enzyme/mtDNA region combinations surveyed. Most variation occurred in the ND1 region, whereas, the ND5/6 and CytB regions showed the least amount of variation. Presumed sequence divergence in the primer recognition sequence of the ND1 and ND5/6 regions in all specimens from Canyon

Creek (population 16, Table 1) prevented amplification of these regions and precluded evaluation of this population in the subsequent analysis.

Geographic Patterns of Mitochondrial DNA Diversity

Analysis of bull trout mtDNA revealed 21 composite haplotypes (hereafter haplotypes), labeled A-U (Table 2). Haplotype-A appeared common to Demming and Brownsworth Creeks from the Klamath River (Table 2). Haplotype-F had a widespread and common distribution throughout the Columbia and upper Snake River drainages. It occurred in 30% of all specimens in the study and in seven of the 15 Columbia River populations (Table 2). No other haplotype occurred at frequencies greater than 9%. Distance and parsimony analyses, described below, were conducted on the 19 mtDNA haplotypes for which complete data were available. Haplotypes, T and U from the Canyon Creek population failed to amplify in the ND1 and ND5/6 regions.

Little geographic structuring was apparent among bull trout mtDNA haplotypes in the distance-based dendrogram (Figure 2). Nevertheless, both Klamath River haplotypes (A and B) clustered together, as did two haplotypes (C and D) that were specific to each of the two populations from the Lower Columbia River. Haplotypes from the latter two populations, Rush Creek in the Lewis River and Jack Creek in the Metolious River, were similar to one another (0.28% sequence divergence; Table 3), but differed from the Klamath River haplotypes, and the common Columbia/Snake River haplotype by approximately 1% sequence divergence. Common haplotypes for the latter two groups (Haplotypes A and F, respectively) differed from one another by 0.48% sequence divergence. Bull trout differed from brook trout by approximately 5% sequence divergence (Table 3).

The Columbia and Snake River populations, except for Rush and Jack Creeks from the lower Columbia River, formed a single cluster that included all remaining haplotypes except Haplotype-K, which occurred in a single specimen from Little Crane Creek in the North Fork of the Malheur River. Haplotype-K appeared to be more similar to Klamath River bull trout haplotypes than Columbia River bull trout haplotypes. Haplotypes within the Columbia River Group differed from one another over a range from 0.02 - 0.66% sequence divergence (Table 3), but showed no particular geographic pattern in their relationships.

Maximum parsimony analysis also showed little geographic pattern among bull trout mitochondrial haplotypes; however, Klamath haplotypes were separated from Columbia River haplotypes. Parsimony analysis showed the two lower Columbia River populations from the Lewis and Metolius Rivers to be sister taxa and divergent from other Columbia River bull trout; however, they did not form a clade that was separated from all other Columbia River populations (Figure 3). Parsimony analysis also separated both haplotypes (J and K) from the Little Crane population in the North Fork of the Malheur River from all other haplotypes observed in Columbia River populations. Although haplotypes J and K did not form a distinct clade with those from the Klamath River populations, they appear to be more closely related to Klamath River bull trout haplotypes than to Columbia River bull trout haplotypes.

MtDNA Diversity Within and Among Populations

Within-population haplotype diversity was generally low among the 17 sample populations with nine populations exhibiting only one mtDNA haplotype, five populations having two mtDNA haplotypes, two populations having three haplotypes, and one population with five haplotypes (see Table 2). Within the upper

Columbia and Snake River clade, two very different patterns of mtDNA diversity were observed. The first pattern revealed little diversity either within or among disjunct populations. Haplotype-F occurred in seven of the thirteen up-river populations and was either the only or the most common haplotype in six of the seven populations.

Specimens from the St. Joe River, the Clark Fork River, and South Gold Creek (a tributary of Lake Pend Oreille) presented a second and markedly different pattern of mtDNA diversity in which high levels of within-population diversity occurred. Fourteen specimens were examined from Medicine Creek in the St. Joe watershed and two mtDNA haplotypes were observed in equal frequency. Both haplotypes were unique to bull trout in the St. Joe River and not observed elsewhere in the study. Five bull trout specimens each were examined from the Clark Fork and South Gold Creek populations with three and five mtDNA haplotypes observed, respectively. Three specimens in the Clark Fork sample had the common Columbia/Snake River haplotype (F). Haplotype-L was shared by one specimen each from the Clark Fork and South Gold Creek samples; whereas Haplotype-M was unique to one specimen from the Clark Fork sample. Haplotypes-N. -O, -P, and -Q were unique to individual specimens from the South Gold Creek sample. The amount of diversity observed in these three populations is unusually high when compared to that observed in most other populations in this study. However, pairwise estimates of genetic divergence among haplotypes F and L-Q averaged 0.23% sequence divergence (range: 0.04 - 0.54%) and were similar to variation observed in other natural salmonid populations, where *in situ* mutations have resulted in the presence of two or more closely related mtDNA haplotypes (Billington and Hebert 1991; Willams et al. in press a).

The high within-population diversity of the Clark Fork and South Gold Creek

samples was surprising because of the limited number of females used in the hatchery matings. The Clark Fork River sample contained progeny of three females and two males taken from the Clark Fork River and the South Gold Creek sample contained progeny from eight females and an unknown number of males collected off spawning redds in South Gold Creek (Joe Chapman, Idaho Department of Fish and Game, personal communication). Therefore, our sample of these two populations may not accurately represent either the total haplotype diversity or the relative frequencies of the different haplotypes within each population.

Discussion

Geographic Patterns of Mitochondrial DNA Diversity

Bull trout in the Columbia River showed little evidence of geographically structured evolutionary divergence among currently isolated populations as inferred from RFLP analysis of mitochondrial DNA fragments. Nevertheless, parsimony and distance analyses of restriction site differences among the 19 mtDNA haplotypes identified three groups of populations: a Klamath River group (two populations), a small lower Columbia River group (two populations) and a larger upper Columbia and Snake River group (13 populations). Haplotypes in the lower Columbia River group (C and D) differed from the Klamath River haplotypes and the common upper Columbia/Snake River haplotype (F) by approximately 0.84% and 0.94% sequence divergence, respectively. This is equivalent to the divergence that occurs between mtDNA haplotypes of coastal and interior forms of cutthroat trout (*O. clarki*) (0.9 -1.2% sequence divergence; Willams et al. unpublished data) and coastal and interior rainbow trout (1.3% sequence divergence; Williams et al. in press a)

MtDNA Diversity Within and Among Populations

Several different patterns of mtDNA diversity are apparent in bull trout at the species level; nevertheless, most populations we surveyed (13 of 17) had only one or two mtDNA haplotypes, a pattern that is common across many species. Our sample sizes were too small to state with certainty that populations which exhibited only one mtDNA haplotype among a total sample of 5-12 specimens were monomorphic for mtDNA variation. Nevertheless in those cases, it is clear we identified the predominant mtDNA haplotype, and if others were present in the populations, they occurred at low enough frequencies to remain undetected in our analysis. This interpretation is consistent with other studies of mtDNA diversity.

Studies of mtDNA diversity in fishes (Billington 1991; Bermingham and Avise 1986), have found that species generally have a few common haplotypes and a substantially larger number of rare haplotypes which are mutational derivatives of the common haplotypes. Thus, natural populations are typically monomorphic or have low levels of mitochondrial DNA diversity that involve closely-related haplotypes unless the population has experienced gene flow with non-native populations (Dowling and Childs 1992). Populations that exhibit high levels of haplotype diversity (Willams et al. in press a) or "non-native" haplotypes (Bermingham et al. 1991; Billington and Hebert 1991) are thought to have experienced gene flow with other genetically divergent populations, either naturally or through man-aided introductions (Bermingham and Avise 1986; Billington and Hebert 1991).

Within the upper Columbia and Snake River clade, two very different patterns of mtDNA diversity were observed. Most of the Columbia and Snake River bull trout populations (8 of 13) had only one or two mtDNA haplotypes and most populations (7

of 13) had Haplotype-F as their predominant haplotype, suggesting that this haplotype was widespread and common in historic Columbia River bull trout populations. This pattern of low within-population haplotype diversity appears to be similar to that observed in interior rainbow trout (Willams et al. in press a) and cutthroat trout populations (Willams et al. unpublished data) in closed basins of the western United States. These latter populations are frequently thought to have been founded from small isolates captured via headwater exchange (Loudenslager and Gall 1980; Behnke 1992) and are likely to have undergone a reduction in mitochondrial haplotype diversity through stochastic lineage extinction processes (Bermingham and Avise 1986; Billington and Hebert 1991). Lack of divergence among most Columbia River populations suggests either recent isolation from one another or that sufficient gene flow occurred among historic populations to prevent different mtDNA haplotypes from becoming predominant or fixed in specific populations. Gene flow among Columbia River populations may have been episodic and related to cyclical climatic and geologic events, such as the advance and retreat of glaciers or the repeated filling and flooding of Pleistocene Lake Missoula (Bretz 1969; Curry et al. 1977). Allozyme data from Leary et al. (1993) suggest a pattern of repeated founder events, bottlenecks, and genetic drift among Columbia River bull trout populations resulting in little genetic variation within populations, but substantial variation among populations. These results are in contrast to the mtDNA results, which show little variation within or among populations. Differences in results between the two studies are likely related to the differing effects that isolation, bottlenecks, and drift can have on the nuclear versus the mitochondrial genomes.

Divergence among populations could result from the stochastic nature of mitochondrial lineage extinctions, as well as the accumulation of random mutations

over evolutionary time. An example of this pattern occurs in bull trout from Rush and Jack Creeks in the lower Columbia and Early Winters Creek in the mid-Columbia which have low within-population diversity — each is monomorphic for a different haplotype — but each has diverged from the common haplotype (F) of most Columbia and Snake River populations (e.g., 1.07%, 0.81%, and 0.36% sequence divergence, respectively).

A second and markedly different pattern of mtDNA diversity was observed among specimens from the St. Joe River and the Lake Pend Oreille drainage (LPOD) in which high levels of within-population diversity occurred. MtDNA diversity in the St. Joe River sample (N=14) with two haplotypes, the Clark Fork River sample (N=5) with three haplotypes, and the South Gold Creek sample (N=5) with five haplotypes, is high when compared to that observed in most other populations in this study. Nevertheless, little genetic divergence appears associated with the high levels of mitochondrial diversity, as all haplotypes observed in the three samples are closely related to one another and include haplotype-F, the haplotype common to upper Columbia and Snake River populations. Several possibilities exist concerning the origin of this high level of mtDNA diversity.

Differences in life history attributes may have contributed to the high mtDNA diversity observed in LPOD bull trout samples. Bull trout in Lake Pend Oreille may have developed distinct natal populations in various spawning streams, each undergoing its own incipient mitochondrial evolution, thus allowing an overall increase in mtDNA diversity in the lake metapopulation. A limited amount of gene flow between spawning populations due to straying would act to increase mtDNA diversity within local populations. Therefore, each stream population should have a predominant haplotype resulting from homing fidelity and lineage extinction

processes, but might also include a range of other closely related haplotypes present at low frequencies, remnants of natal infidelity, as well as further *in situ* mutations of the mitochondrial genome.

A second possibility is that the founding inoculum of bull trout into the Lake Pend Oreille drainage was large enough to include numerous mtDNA haplotypes and has remained large enough so that lineage extinction processes have been buffered. Thus, mutational changes present or occurring were retained in the system, and the existing diversity seen in the reduced populations today are a reflection of historical population sizes. Under this scenario, one would predict that the diversity in the lake population would be similar to diversity within the spawning streams, with several haplotypes shared among different spawning populations. Now that bull trout populations are greatly reduced, one would predict that lineage extinction should become a dominant process in the smaller sized populations, and losses of extant mtDNA diversity will be the ultimate result.

Salmonid populations with high levels of haplotype diversity (Willams et al. in press a) are thought to have experienced gene flow with other populations, either naturally or as a result of human activities, such as introduction of non-native salmonids (Bermingham and Avise 1986; Billington and Hebert 1991). Thus, a third possibility for the high levels of mtDNA diversity observed in LPOD bull trout is that either non-native bull trout have been introduced in the Lake Pend Oreille drainage, or that migratory bull trout from elsewhere in the Columbia system have entered the lake and with them came the increased mtDNA diversity.

Introductions of non-native bull trout do not appear to have been a factor. No stocking of bull trout has occurred in Lake Pend Oreille (Mike Larkin, Hatchery Program Manager, Idaho fish and Game, personal communication); however,

extensive stocking of Alaskan dolly varden occurred between 1971 - 1974. Leary et al. (1993) found no evidence of hybridization with dolly varden among bull trout samples from the Clark Fork River and South Gold Creek. Although mtDNA analysis of these same specimens revealed unusually high within-population diversity, none of the observed mtDNA haplotypes appeared to be non-native ones, which usually differ from indigenous ones by a much larger degree of divergence than *in situ* mtDNA variation (Billington and Hebert 1991; Williams et al. in press a). Haplotypes in the Clark Fork and South Gold Creek samples are closely related to one another, as well as to the common Columbia/Snake River haplotype (F). Mean sequence divergence among the LPOD samples and haplotype-F was 0.23% (range: 0.04 - 0.54%), which compares to *in situ* mtDNA variation in interior rainbow trout (Williams et al. in press a) and cutthroat trout (Williams et al. unpublished data). In the latter two groups, non-native mtDNA haplotypes differed from native haplotypes by approximately 1.0 - 1.5% sequence divergence.

An increase in overall mtDNA diversity in bull trout in the Lake Pend Oreille system may have been the result of downstream migration by individuals from recently diverged bull trout populations in the upper Clark Fork and Flathead Rivers into Lake Pend Oreille. Historical information summarized by Pratt and Huston (1993) suggests that the upper Clark Fork and Flathead River systems contained several different stocks of bull trout that were not continuously distributed. Allozyme data from Kanda et al. (in press) show genetic differences in bull trout from the major drainage forks of the Flathead River. Approximately 80% of the allozyme variation that exists among bull trout in the Flathead system could be attributed to differences that occurred between bull trout from different drainages. The remaining 20% occurred between bull trout populations within drainages. Thus, it is possible to

speculate that bull trout from different drainages may also have divergent mtDNA haplotypes. Kanda and associates are currently investigating this hypothesis.

Numerous dams on the upper Columbia, Pend Oreille, Clark Fork, and Flathead Rivers have fragmented a large system that was characterized by bull trout with migratory (adfluvial) life history patterns (Pratt 1985; Bjornn 1987; Fraley and Shepard. 1989; Pratt 1992), Historically, bull trout in the LPOD may have had higher levels of mtDNA diversity than populations lower in the Columbia River system; however, fragmentation of the Clark Fork and Flathead Rivers through dam construction has probably served to further increase mtDNA diversity of bull trout in Lake Pend Oreille and its direct tributaries. In an adfluvial life history, juvenile salmonids migrate from natal areas downstream into large rivers or lakes, where they mature before migrating back to their natal streams to reproduce. In the fragmented Clark Fork and Flathead systems, juveniles are able to pass downstream into Lake Pend Oreille during spring flows, but are unable to migrate upstream to their natal streams in the fall due to blockage by dams, many of which lack or have inefficient bypass facilities (Pratt and Huston 1993). Thus, downstream drift from several source populations (e.g., Flathead, upper Clark Fork, Bitterroot) may have introduced several different mtDNA haplotypes into Lake Pend Oreille. Adult fish unable to migrate back to their natal spawning areas would likely seek secondary non-natal areas for spawning, thus increasing the mtDNA diversity in local populations.

Resolution of the source or cause of the increased mtDNA diversity observed in the Lake Pend Oreille bull trout populations is beyond the data base generated in this study. However, the proposed causal factors could be investigated with an expanded data base. It is likely that all three hypothesized causes have partial responsibility.

Clearly, genetic diversity needs to be examined in more bull trout populations from the upper basin (Clark Fork River, Flathead River) and from other large lake systems, such as Flathead Lake and Lake Billy Chinook in central Oregon. If similar patterns of diversity occur there as in the Lake Pend Oreille system, and the primary causal factors can be deciphered from a larger data base, then broader generalizations about mitochondrial DNA diversity patterns cans be generated.

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Appendix 1. Fragment patterns for restriction enzymes (by amplification region) where polymorphisms were observed in mtDNA from bull trout from the Klamath and Columbia Rivers.

ND1 Region

Cfo I

C	D
781	781
329	329
272	272
252	---
---	218
196	196
166	166
1996	1962

Hae III

A	B	E	F	G
---	---	---	---	775
609	609	609	609	609
487	487	487	487	---
318	---	---	---	---
---	---	---	297	---
---	---	279	---	---
---	238	---	---	---
200	200	200	200	200
165	165	165	165	165
141	141	141	141	141
1920	1840	1881	1899	1890

Hpa II

B	C	D	F
---	---	---	1428
1027	1027	1027	---
399	---	399	---
---	365	---	---
---	260	260	---
225	---	---	225
186	186	186	186
161	161	---	161
---	---	90	---
---	---	70	---
1998	1999	2032	2000

Rsa I

B	C	D	E
440*	440*	440*	440*
351	351	---	---
---	---	323	323
---	307	---	307
280	280	280	---
275	---	275	---
240	240	240	240*
2026	2058	1998	1990

Appendix 1. (continued)

ND1 Region				ND2 Region										
Dde I				*Sau3A* I				*Hpa* II				*Mse* I		
A	B	C	D	C	D	E	F	B	C	D	F	B	C	D
---	---	826	---	---	---	2200	---	---	---	1301	---	---	---	1547
---	675	---	---	---	1179	---	1179	1034	1034	---	1034	1171	1171	---
629	---	---	---	735	---	---	---	---	---	---	460	376	---	---
---	---	---	525	480	480	---	480	389	---	---	---	242	242	242
504	504	504	504	369	369	---	369	258	258	258	258	207	207*	207
271	271	271	271	302	---	---	---	232	232	232	232	202	202	202
229	229	229	229	---	---	---	269	222	222*	222	222	---	150	---
210*	210*	210	210	223	223	---	---	---	192	192	---	2198	2179	2198
2053	2099	2040	2010	2109	2251	2200	2297	2135	2160	2205	2206			

Appendix 1. (continued)

ND5/6 Region

Alu I B	*Alu* I C	*Hinf* I B	*Hinf* I C	*Rsa* I D	*Rsa* I C	*Rsa* I E
---	777	980	980	1183	1183	---
683	683	387	387	889	889	889
550	550	357	357	---	---	795
527	---	291	---	---	417	417
387	387	---	248	---	---	360
210	---	226	226	327	---	---
2357	2397	199	199	2399	2489	2461
		2440	2397			

Cytochrome B Region

Alu I C	*Alu* I D	*Hae* II C	*Hae* II D	*Hae* II E	*Dde* I B	*Dde* I C
447	447	---	992	---	613	---
369	---	496	---	496	---	450
---	257	280	---	280	277	277
225	225	161	161	161	159	159*
---	153	---	---	156	151	151
148	148	137	137	---	1200	1196
1189	1230	107	---	107		
		1181	1290	1200		

Appendix 2. Restriction sites of fragment patterns from restriction enzymes where polymorphisms were observed among mtDNA from bull trout from the Klamath and Columbia Rivers and brook trout. Restriction sites are listed by amplification region and restriction enzyme. Fragment patterns are identified by letters that correspond to fragment patterns (Appendix 1) and are included in the composite haplotypes (Table 2).

Enzyme	Amplified Region			
Pattern	ND1	ND2	ND5/6	CytB
Alu I				
A	111100110	100111101	10000010111	00111111
B	111011111	011111011	00101101011	01101101
C	110011111		01101001001	11001001
D	111010111		00011101011	10011011
Cfo I				
A	0110101001	111	011101	011111
B	0110001001		101001	101011
C	0111101011		011011	
D	1010101011			
E	1010100111			
F	1111000000			
G	1110010000			

Appendix 2. (continued)

Enzyme Pattern	Amplified Region ND1	ND2	ND5/6	CytB
Hae III				
A	01100100111	1001101101	010011000110	0011110011
B	01100010111	1011001101	010011010100	0110010011
C	01001010111	0111011111	101100011001	0110001011
D	01010100111	0111001101	001100111001	1000001011
E	01100011111			0110001101
F	01100101111			
G	11000010111			
*Hin*f I				
A	1110	0111100	0011110011	10001001
B	1101	0111001	1011100101	01001111
C		1010010	1011001101	00111111
D			0111100101	
Mbo I				
A	1111111	010011000	001110111	0110101
B		010101000	100010111	1001011
C		001010111	010011100	
D		010010101		
E		100000000		
F		010010110		
G		011000101		

Appendix 2. (continued)

Enzyme	Amplified Region			
Pattern	ND1	ND2	ND5/6	CytB
Msp I				
A	001010101100	00011011010	1101	011001011
B	010010101100	00100101110	1011	000111111
C	010001101100	00100001111		101101011
D	010010011011	01000001111		
E	000111101100	10000001110		
F	110000101100	00110001110		
Rsa I				
A	110101	1000010	1000001010	1101101
B	110011	0101010	1000010010	1110011
C	110111	0011011	0110000101	1101011
D	101011	0011111	0110100000	
E	101101		0011100010	
F			1100000000	
G			0100000100	
Dde I				
A	001011111	100100011	1010101	100101
B	010011111	001011111	0101111	010011
C	100011110	110001001		001011
D	000111110			

Table 1. Sample populations of bull trout by location, major river drainage, state, and sample size.

			N
I. Klamath River Drainage			
1) Brownsworth Creek	S. Fk. Sprague River	OR	5
2) Demming Creek	S. Fk. Sprague River	OR	5
II. Columbia River Drainage			
3) Rush Creek	Lewis River	WA	10
4) Jack Creek	Metolius River	OR	10
5) Early Winters Creek	Methow River	WA	10
6) Granite Boulder Creek	John Day River (Mid. Fk.)	OR	8
7) Deardorf Creek	John Day River (Mainstem)	OR	7
8) S. Fk. Catherine Creek	Grand Ronde River	OR	10
9) Big Creek	Malheur River (Mid. Fk.)	OR	3
10) Little Crane Creek	Malheur River (N. Fk.)	OR	6
11) Clark Fork River	Clark Fork River	ID	5
12) South Gold Creek	Lake Pend Oreille	ID	5
13) Profile Creek	Salmon River (S. Fk.)	ID	12
14) Queen's River	Boise River (Mid. Fk.)	ID	1
15) Sawmill Creek	Little Lost River	ID	10
16) Canyon Creek	Payette River (S. Fk.)	ID	12
17) Medicine Creek	St. Joe River	ID	14
		N = 133	

Table 2. List of 21 MtDNA composite haplotypes from bull trout specimens, showing sample locations, haplotype designation, haplotype frequency, and composite haplotype. Fragment patterns for each amplified region are denoted by letters and represent patterns detected with the following restriction enzymes: *Alu* I, *Cfo* I, *Hae* III, *Hinf* I, *Mbo* I, *Msp* I, *Rsa* I, *Sau*3A I, *Dde* I, and *Hpa* II. *Mbo* I and *Msp* I are isoschizomers of *Sau*3A I and *Hpa* II, respectively.

	Haplotype		Amplified Regions			
Location	des.	freq.	ND1	ND2	ND5/6	CytB
Brownsworth	A	5	BDAAACCACC	BACCDBBDBB	CBCBCBDCBB	CBCBBBBBBB
Demming	B	4	BDAAABCACB	BACCDBBDBB	BBCBCBDCBB	CBCBBBBBBB
	A	1	BDAAACCACC	BACCDBBDBB	CBCBCBDCBB	CBCBBBBBBB
Rush	C	7	BEFAADEADD	BACCFDBFBD	BBCCCBECBB	CBCBBBBBBB
Jack	D	5	BEEAADEACD	BACCFCBFBC	BBCBCBDCBB	CBCBBBBBBB
Early Winters	E	6	BEEAADDAAD	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
Granite Boulder	F	3	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
Deardorf	F	3	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
	G	1	BEDAADDAAD	BACCEBBEBB	BBCBCBCCBB	CBCBBBBBBB
	H	1	BEBAADDAAD	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB

Table 2: (continued)

	Haplotype		Amplified Regions			
Location	des.	freq.	ND1	ND2	ND5/6	CytB
Catherine	F	6	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
Big Creek	F	1	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
	I	1	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBDBBBBBCB
Little Crane	J	4	BDAAACCACC	BACCEBBEBB	BBCBCBDCBB	CBCBBBBBBB
	K	1	BDBAABCACC	BACCEBBEBB	CBCBCBDCBB	CBCBBBBBBB
Clark Fork	F	3	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
	L	1	BEBAABDAAB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
	M	1	BEEAABDAAB	BACCCBBCBB	BBCBCBDCBB	DBCBBBBBBB
South Gold	L	1	BEBAABDAAB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
	N	1	BEEAABDAAB	BACCCBBCBB	BBCBCBDCBB	CBEBBBBBBB
	O	1	BDBAABBABB	BACCCBBCBB	BBCBCBDCBB	CBEBBBBBBB
	P	1	BDBAABBABB	BACCCBBCBB	BBCBCBDCBB	CBCBBBBBBB
	Q	1	BDBAABBABB	BACCCBBCBB	BBCBCBDCBB	DBCBBBBBBB

Table 2: (continued)

Location	Haplotype des.	Haplotype freq.	ND1	ND2	ND5/6	CytB
			Amplified Regions			
Sawmill	F	6	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
Profile	F	6	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBCBBBBBBB
Queens	I	1	BDBAABBABB	BACCCBBCBB	BBCBCBCCBB	CBDBBBBBBB
Medicine	R	7	BEGAAFDAAB	BBCCCFBCBB	BBCBCBCCBB	CBCBBBBBBB
	S	7	BEBAABDAAB	BBCCCFBCBB	BBCBCBCCBB	DBCBBBBBBB
Canyon	T	8	----------	BACCCBBCBB	----------	CBCBBBBBBB
	U	2	----------	BBCCCFBCDB	----------	CBCBBBBBBB
Brook Trout	BRK		AAAAAAAAAA	AAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA

Table 3. Percent sequence divergence (d x 100) among 19 mitochondrial DNA haplotypes among 16 populations of bull trout from the Klamath and Columbia River drainages. Haplotypes are listed in alphabetical order (see Table 2 for association of haplotype letter with sample populations). Frequency of the haplotype within the total study sample is shown in parentheses. Also listed is the mtDNA haplotype from a brook trout (Brk) that was used as an outgroup for the phylogenetic analysis.

Table 3. continued.

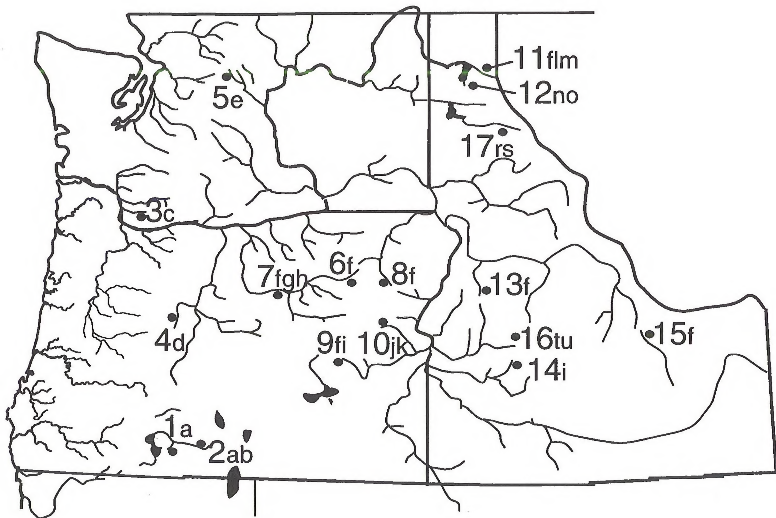
haplotype	(frequency)	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q		
B	(5)	0.15																		
C	(9)	1.04	0.86																	
D	(7)	0.82	0.65	0.28																
E	(8)	0.83	0.66	0.71	0.45															
F	(41)	0.48	0.32	1.07	0.81	0.36														
G	(1)	0.80	0.64	0.73	0.48	0.02	0.34													
H	(1)	0.86	0.69	0.79	0.53	0.14	0.48	0.12												
I	(2)	0.60	0.44	1.20	0.93	0.47	0.10	0.45	0.59											
J	(1)	0.44	0.28	1.04	0.77	0.59	0.21	0.57	0.44	0.32										
K	(5)	0.10	0.26	1.12	0.90	0.90	0.55	0.88	0.75	0.67	0.33									
L	(2)	0.57	0.41	0.98	0.71	0.23	0.12	0.21	0.34	0.23	0.35	0.65								
M	(1)	0.89	0.73	0.77	0.52	0.06	0.43	0.08	0.21	0.54	0.66	0.98	0.29							
N	(1)	0.57	0.41	0.93	0.67	0.32	0.25	0.34	0.48	0.36	0.35	0.65	0.12	0.38						
O	(1)	0.46	0.30	1.05	0.79	0.47	0.10	0.45	0.59	0.21	0.19	0.53	0.23	0.54	0.14					
P	(1)	0.41	0.26	1.00	0.74	0.43	0.06	0.41	0.55	0.17	0.15	0.49	0.19	0.49	0.19	0.04				
Q	(1)	0.64	0.48	1.05	0.78	0.29	0.19	0.27	0.41	0.30	0.41	0.72	0.06	0.23	0.19	0.30	0.25			
R	(7)	0.69	0.57	1.10	0.83	0.39	0.28	0.50	0.37	0.39	0.77	0.51	0.15	0.45	0.28	0.39	0.35	0.21		
S	(7)	0.71	0.55	1.07	0.81	0.36	0.26	0.48	0.34	0.37	0.79	0.48	0.13	0.30	0.25	0.37	0.32	0.06	0.14	
Brk		4.89	4.66	5.34	5.41	5.48	4.98	5.46	5.45	5.04	4.89	4.98	5.07	5.33	5.15	4.96	4.92	4.92	5.18	4.87

Figure Legends

Figure 1. Map of Columbia River Basin and locations where bull trout populations were sampled. Population numbers and names are listed in Table 1. Lower case subscripts following population numbers denote mtDNA haplotypes observed in each population (listed in Table 2).

Figure 2. Distance phylogram showing relationships among 19 bull trout mtDNA haplotypes derived from 14 populations from the Columbia River drainage and two populations from the Klamath River drainage. Brook trout was used as an outgroup.

Figure 3. Parsimony cladogram showing relationships among 19 bull trout mtDNA haplotypes derived from 14 populations from the Columbia River drainage and two populations from the Klamath River drainage. Brook trout was used as an outgroup.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5e
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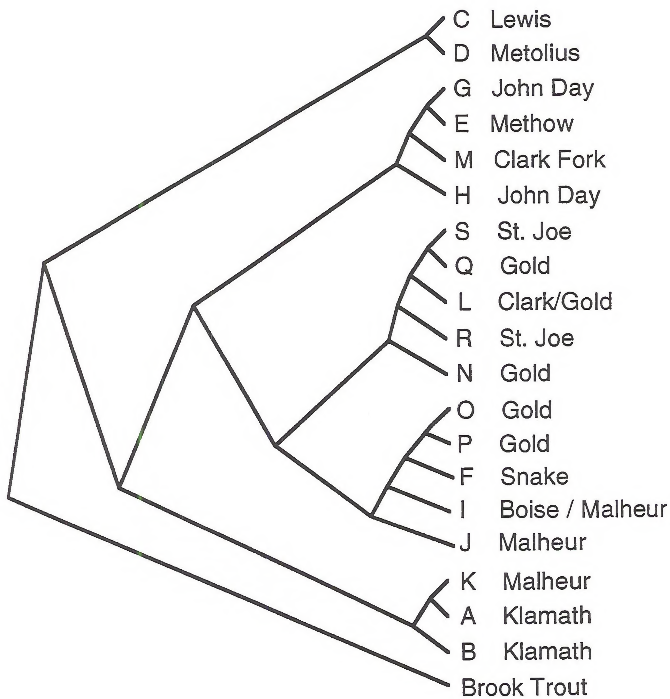
3c
6f
7fgh
8f
13f
4d
9fi
10jk
16tu
15f
14i
1a
2ab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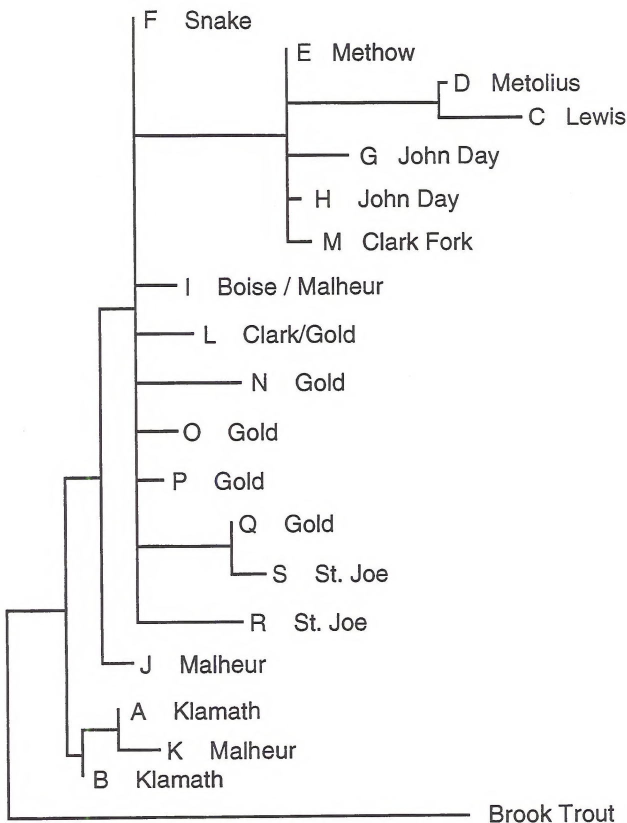
D Metolius
G John Day
E Methow
M Clark Fork
H John Day
S St. Joe
Q Gold
L Clark/Gold
R St. Joe
N Gold
O Gold
P Gold
F Snake
I Boise / Malheur
J Malheur
K Malheur
A Klamath
B Klamath
Brook Trout



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